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(54) GENE THERAPY FOR CARDIOMYOPATHY

(57) This invention enables the repair of cardiac function by noninvasive administration of an HGF gene in the form of Sendai virus (HVJ)-liposome into the affected cardiac muscle, thereby inducing angiogenesis of the cardiac muscle layer and repressing fibrosis.

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Description

Technical Field

[0001] The present invention relates to a method of gene therapy for treating myocardiopathy by noninvasive administration of an HGF (hepatocyte growth factor) gene and therapeutic agents used therefor. More specifically, the present invention relates to a method of gene therapy for treating myocardiopathy by noninvasive administration of an HGF gene into the cardiac muscle, especially to a method of gene therapy that more efficiently treats heart disease, such as cardiomyopathy, angina pectoris and heart failure, by injecting an HGF gene into the affected part of cardiac muscle under the usage of echo, and to therapeutic agents used therefor. Moreover, the present invention relates to a method of gene therapy which is applicable to genes other than HGF genes and that consists of administering genes to the affected part of tissue noninvasively under the usage of echo.

Background Art

[0002] In spite of the recent striking technical improvements in the medical field, many problems remain unsolved. The problem of myocardiopathy is one of the important unsolved subjects.

[0003] Myocardiopathy is a general name for diseases attributable to organic and functional abnormalities of the cardiac muscle. For example, cardiomyopathy is classified into secondary cardiomyopathy, which occurs in sequence to hypertension, dysbolism, ischemic disease and such, and idiopathic cardiomyopathy (ICM), which occurs without any distinct fundamental disease. Hypertrophic cardiomyopathy (HCM) is classified as an ICM, whose cause of disease is most revealed at the genetic level. In half the numbers of patients with HCM, familial history following autosomal dominant heredity is recognized. Linkage analysis of such family lines, with multiple patients as the object, revealed 5 causal loci so far and the causal gene itself is specified in 4 of them.

[0004] Many cases of dilated cardiomyopathy (DCM) occur independently, but familial history is recognized in 20% of the cases. Linkage analysis of such family lines, with multiple patients as the object, revealed 7 types of causal loci (causal genes are unknown).

[0005] Regarding myocardiopathy, research is in progress to specify causal gene and to reveal the mechanism underlying the start of disease. So far, no concrete action for gene therapy has been done.

[0006] On the other hand, the rapid progress lately in molecular biology has made it possible to activate cellular function by gene transfer methods and various attempts have been made. In particular, there are some reports for gene transfer methods to the heart, like intravenous drip (J.Clin.Invest., 90, 626-630(1992)), direct injection (Circulation, 82, 2217-2221 (1990); Circulation, 90, 2414-2424(1994)) or coronary diffusional infusion method that utilizes the plasmid as it is (J.Thorac. Carduivasc.Surg., 109, 716-720(1995)) and so on, but were far from noninvasive concrete treatment.

Disclosure of the Invention

[0007] The object of this invention is to provide a noninvasive treatment for myocardiopathy, for which effective treatment is currently unknown, and therapeutic agents used therefor. That is, the present invention relates to a method of gene therapy for treating myocardiopathy by noninvasive administration of an HGF gene and therapeutic agents used therefor. More specifically, the present invention relates to a method of gene therapy for treating myocardiopathy by noninvasive administration of an HGF gene into the cardiac muscle, especially to a method of gene therapy for treating myocardiopathy that more efficiently treat a heart disease, such as cardiomyopathy, angina pectoris and heart failure, by injecting an HGF gene to the affected part of cardiac muscle under the usage of echo, and to therapeutic agents used therefor. Moreover, the present invention relates to a method of gene therapy which is applicable to genes other than HGF genes and that consists of administering genes to the part of affected tissue noninvasively under the usage of echo.

[0008] Present inventors investigated to find out that effective results are obtained by using an HGF gene as the gene and noninvasively infusing directly to the affected part of cardiac muscle layer. That is, present inventors found out that it is effective to infuse HGF gene to the affected part of cardiac muscle optically using echo without incision of the affected part or thoracotomy. Since this method is a noninvasive treatment, it is possible to administer the present gene repeatedly, according to the condition, and therefore it is possible to treat myocardiopathy efficiently.

[0009] Present inventors newly discovered that effective treatments can be done by infusing genes to the affected part optically using echo and showed that the method of the present invention enables genetic. treatment of various organ-specific disease.

[0010] For example, in the case where the HGF gene is used, according to the present invention, it is possible to treat various organ-specific diseases like pulmonary fibrosis, cirrhosis, hepatic fibrosis and so on. Furthermore, genes other than the HGF gene are also effective in the method of the present invention above.

[0011] Thus, the outline of the present invention is as follows:

(1) a therapeutic agent for myocadiopathy used for noninvasive administration comprising a hepatocyte growth factor (HGF) gene as the effective ingredient;

(2) the therapeutic agent of (1). which is used for administration of the HGF gene into the cardiac

muscle;
(3) the therapeutic agent of (1) or (2), wherein the HGF gene is in the form of Sendai virus (HVJ)-liposome;
(4) the therapeutic agent of (2) or (3) , which is used for noninvasive administration to the affected part of the cardiac muscle under the usage of echo;
(5) the therapeutic agent of any of (1) to (4), which is to be administered at least 8 times, once a week;
(6) the therapeutic agent of any of (1) to (5), wherein at least 10 μg of the HGF gene is used;
(7) the therapeutic agent of any of (1) to (6), wherein the myocardiopathy is selected from the group consisting of cardiomyopathy, angina pectoris and heart failure;
(8) a gene therapy agent used for noninvasive administration of a gene into an affected part of a tissue under the usage of echo, which comprises genes effective for the treatment of a disorder as the effective ingredient;
(9) the gene therapy agent of (8), wherein the affected part of the tissue is the cardiac muscle;
(10) the gene therapy agent of (8) or (9), wherein the gene is an HGF gene;
(11) a method for gene therapy for myocardiopathy, which comprises the noninvasive administration of an HGF gene into the cardiac muscle of a mammal, including a human;
(12) the method for gene therapy of (11), wherein the HGF gene is in the form of Sendai virus (HVJ)-liposome;
(13) the method for gene therapy of (11) or (12), wherein the HGF gene is administered noninvasively to a part of an affected cardiac muscle under the usage of echo;
(14) the method for gene therapy of any of (11) to (13), wherein the HGF gene is administered at least 8 times, once per week;
(15) the method for gene therapy of any of (11) to (14), wherein the myocardiopathy is selected from the group consisting of cardiomyopathy, angina pectoris and heart failure;
(16) a method for gene therapy. which comprises the noninvasive administration of genes effective for the treatment of a disorder into an affected part of a tissue under the usage of echo;
(17) the method for gene therapy of (16) , wherein the affected tissue is the cardiac muscle;
(18) the method for gene therapy of (16) or (17), wherein the gene is an HGF gene;
(19) use of an HGF gene for the production of a therapeutic agent for myocardiopathy used for noninvasive administration;
(20) the use of (19), wherein the HGF gene is in the form of Sendai virus (HVJ)-liposome;
(21) the use of (19) or (20), wherein the therapeutic agent is a therapeutic agent used for the noninvasive administration of the HGF gene to an affected part of the cardiac muscle under the usage of echo;
(22) the use of any of (19) to (21), wherein the myocardiopathy is selected from the group consisting of cardiomyopathy, angina pectoris and heart failure;
(23) use of a gene for the production of a gene therapy agent used for the noninvasive administration of genes effective for the treatment of a disorder into an affected part of a tissue under the usage of echo;
(24) the use of (23), wherein the affected tissue is cardiac muscle; and
(25) the use of (23) or (24), wherein the gene is an HGF gene

Brief Description of the Drawings

[0012] Figure 1 is a graph showing that gene transfer under usage of echo is possible. It is proven by the high activity rate of luciferase in cardiomyopathy guinea pig, in which luciferase as the reporter gene is introduced to the heart using HVJ.

[0013] Figure 2 is a graph showing the result of a comparison between an HGF gene and a control by measuring cardiac capillary vessel density by ALP (alkaline phosphatase) staining.

[0014] Figure 3 is a graph showing the result of a comparison of the amount of cardiac bloodstream between an HGF gene group, a control group and a non-treated group by evaluation with a laser Doppler imager (LDI).

[0015] Figure 4 is a graph showing the result of a comparison of the distribution density of fibrosis of the heart by measurement using Masson staining.

Best Mode for Carrying out the Invention

[0016] As used herein, "HGF gene" means a gene that can express HGF (the HGF protein) . Such genes include genes with deletion of a part of the gene sequence, substitution by another base of the gene sequence, insertion of other base sequence, or binding of bases to the 5' terminus and/or 3' terminus, so long as the expressed polypeptide thereof has substantially the same effect as HGF. For example, HGF genes described in Nature 342:440(1989); Japanese Patent No., 2777678; Biochem.Biophys.Res.Commun. 163:967 (1989); and Biochem.Biophys.Res.Commun. 172:321 (1990) are included. These genes can be used in the present invention.

[0017] The base sequence of the HGF gene (the cDNA encoding HGF) of the present invention has been described in the above literature and is also registered with databases, such as Genbank. Thus, based on such sequence information, a suitable DNA portion is used as a PCR primer; for example, by performing an RT-PCR reaction on mRNA derived from the liver or leukocytes, cDNA of HGF can be cloned. Such cloning can easily be performed by a person skilled in the art according to a basic textbook , such as Molecular Cloning

2nd Ed., Cold Spring Harbor Laboratory Press (1989). Modification and such of the HGF gene can be also readily done by a person skilled in the art according to the above basic textbook.

[0018] Subsequently, methods of gene transfer, dosage forms, dose and the like for use in gene therapy of the present invention are explained.

[0019] The dosage form of a gene therapy agent comprising the above gene as an effective ingredient to be administered to patients are roughly classified into two groups: one is the case in which a nonviral vector is used, and the other is in which a viral vector is used. Methods for preparation and administration thereof are explained in detail in experimental manuals (Supplement of Experimental Medicine, Basic Technology in gene therapy, Yodosha (1996); Supplement of Experimental Medicine,. Experimental Methods in Gene Introduction and Expression Analysis, Yodosha (1997); Handbook for Development and Research of Gene Therapy, Japan Society of Gene Therapy ed., NTS (1999)). Specifics are explained below.

A. Usage of a nonviral vector

[0020] A recombinant expression vector, in which a gene of interest has been integrated into a commonly used gene expression vector, may be used to introduce the gene of interest into cells or tissue by the following method etc.

[0021] Illustrative methods of gene transfer into cellsinclude the lipofection method, calcium phosphate co-precipitation method, DEAE-dextran method, direct DNA introduction methods using micro glass tubes, and the like.

[0022] Regarding methods of gene transfer into the tissue, the recombinant expression vector may be incorporated into the cell by subjecting it to any method, such as the gene transfer method with internal type liposome, method of gene introduction with electrostatic type liposome, HVJ-liposome method, improved HVJ-liposome method (HVJ-AVE liposome method), receptor-mediated gene introduction method, method of introducing DNA molecules together with carriers (metal particles) by a particle gun, method of directly introducing naked-DNA, method of introduction with positively-charged polymers and the like.

[0023] Among them, the HVJ-liposome is a fusion product prepared by enclosing a DNA into a liposome made of lipid bilayer, which is fused to inactivated Sendai virus (Hemagglutinating virus of Japan: HVJ). The HVJ-liposome method is characterized by very high fusing activity with the cell membrane as compared to the conventional liposome method, and is a preferred mode of introduction. For the method of preparing HVJ-liposome, see, the literature for details (Separate volume of Experimental Medicine, Basic Technology in gene therapy, Yodosha (1996); experimental Methods in Gene Introduction and Expression Analysis, Yodosha (1997); J Clin.Invest. 93:1458-1464(1994); Am.J.Physiol. 271: R1212-1220 (1996)) and the like, and experimental examples described below for details.

[0024] In particular, the Z strain (available from ATCC) is preferred as the HVJ strain, but other HVJ strains (for example, ATCC VR-907 and ATCC VR-105) may also be used.

[0025] Furthermore, the method of directly introducing naked-DNA is the most simple method among the methods describer above, and in this regard a preferred method of introduction.

[0026] Expression vectors as used herein may be any expression vectors so long as they permit the *in vivo* expression of the gene of interest. Examples include expression vectors such as pCAGGS (Gene 108:193-200 (1991)), pBK-CMV, pcDNA3.1, pZeoSV (Invitrogen, Stratagene) and the like.

B. Usage of a viral vector

[0027] Representative methods that use viral vectors include those using viral vectors such as recombinant adenovirus, retrovirus and the like. More specifically, the gene of interest can be introduced into a DNA virus such as detoxified retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, poxvirus, poliovirus, Sindbis virus, Sendai virus, SV40, human immunodeficiency virus (HIV) and the like, which is then infected to the cell to introduce the gene into the cell.

[0028] Among the above viral vectors, the efficiency of infection of adenovirus is known to be much higher than that of other viral vectors. In this regard, it is preferred to use an adenovirus vector system.

[0029] As methods of introducing a gene therapy agent into a patient, there are *in vivo* methods, which permit direct introduction of the gene therapy agent into the body, and *ex vivo* methods , in which certain cells are removed from human, to which the gene therapy agent is introduced and which are returned into the body thereafter (Nikkei Science, April 1994 issue pp.20-24; Monthly Yakuji, 36(1): 23-48 (1994); Supplement To Experimental Medicine 12 (15) (1994); Handbook for Development and Research of Gene Therapy, NTS (1999)). According to the present invention, the *in vivo* method is preferred

[0030] Dosage forms may take various forms according to various administration regimens described above (for example, liquids). When, for example an injection containing the gene as an effective ingredient is to be used, said injection may be prepared by dissolving the effective ingredient(s) into a standard solvent (a buffer such as PBS, physiological saline, sterile water, etc.). The injection liquid may then be filter-sterilized with filter as needed and then filled into sterilized containers. Conventional carriers and so on may be added to the injection. Liposomes, such as HVJ-liposome, may take the form of suspensions, frozen formulations, centrifugation-concentrated frozen formulations, and the like.

[0031] In addition to the HGF gene introduced in this invention, it is possible to use endogenous cardiac muscle protective factors or regeneration factors against cardiac muscle. For example, it is reported that factors, such as TGF-β and heat shock protein (HSP) expressed highly during damage of the cardiac muscle, reduce myocardiopathy and are engaged in the repair of cardiac muscle. Therefore, it is possible to use the genes encoding them. Moreover, growth factors, such as EGF, are reported to repair cell damage in various tissues and genes encoding them can be also used. In addition to these cardiac muscle protective factors and regeneration factors, factors related to protection and regeneration of the cardiac muscles can be utilized.

[0032] According to the invention, it is possible to deliver the protein of interest to damaged cells, such as cardiac muscle cells, by introducing an HGF gene, alone or together with other genes, to the cardiac muscle cell of the heart and highly expressing them. This enables activation of repair and regeneration of the damaged cardiac muscle and such, and recuperation of the cardiac function involved in myocardiopathy. Hence, the gene therapy agent of this invention can be applied to patients with critical cardiomyopathy, and offers remedy for patients for whom no options, other than heart transplantation, are left.

[0033] Moreover, the therapeutic agent of this invention can be applied not only to patients with severe cardiomyopathy but also to patients with progressive mild cardiomyopathy. It is applicable to patients of myocardiopathy-like angina pectoris and heart failure as well.

[0034] Proper methods and sites for administration adequate for the disease or symptom to be treated are selected for the gene therapy agent of this invention. Cardiac muscle (affected part of the cardiac muscle) is a preferable administration site. As to the administration methods, parenteral administration methods are preferred.

[0035] Examples of parenteral administration methods include administration by noninvasive catheter, noninvasive injector and so on. More preferred are administration methods which utilize noninvasive catheter, noninvasive injector and such under the usage of echo. As a method using noninvasive catheter, for example, methods like injecting HGF genes directly can be indicated.

[0036] Dosage of the therapeutic agent of this invention varies depending on the symptoms of the patient but HGF genes 0.0001 mg to 100mg, preferably about 0.001 to 10 mg per adult patients can be defined.

[0037] When the HVJ-liposome form is chosen, HGF genes of a range of about 1 to about 4000 μg, preferably about 10 to about 400 μg per adult patient is selected.

[0038] The therapeutic agent of this invention is suited for administration once every few days or every few weeks, and administration once per week is preferred.

[0039] Frequency of administration is to be selected depending on the symptoms of the patients. In compliance with the object of the treatment, plural administration is suitable, and preferably administration of 8 times can be indicated.

[0040] Further to the present invention, a new gene therapy method and therapeutic agent used therefor, including noninvasive administration of therapeutically effective gene for the treatment of the disorder to the affected tissue site under the usage of echo, is presented. That is, it was revealed for the first time that effective treatments can be achieved visually by administering directly the gene to the affected tissue under the usage of echo. According to the therapeutic treatment of the invention, genes are administered noninvasively and therefore desired genes can be administered as much as the condition demands, which is advantageous as compared to former methods. Gene therapy methods of this invention can be applied to any genes, in addition to HGF gene. This gene therapy method of the invention is particularly effective when applied to the affected site of cardiac muscle. Genes administered in such situations include the HGF gene, TGF-β gene, HSP gene, VEGF gene, FGF gene, EGF gene and so on.

[0041] The present invention will now be specifically explained with reference to the following examples. It should be noted, however, that the present invention is not limited by these examples in any way.

Materials and Methods

Experimental Animals

[0042] Hamster model for cardiomyopathy (cardiomyopathy hamster; Bio14.6) was purchased from Oriental Yeast.

HGF gene

[0043] Human HGF gene was cloned from human HGF cDNA (Japanese Patent No.2777678) according to a conventional method and was inserted into the expression vector pcDNA (Invitrogen).

Experimental Procedure

[0044]

1. Reporter gene luciferase was introduced into the cardiomyopathy hamster by HVJ liposome under the usage of echo. A week later, the activity of the luciferase was measured. Animals into which PBS was introduced alone under the usage of echo were used as the control. Luciferase activity was measured by a luminometer (LamatLB9507(BERTHOLO)).

2. Under the usage of echocardiogram (MD500. YOKOKAWA-GE), HVJ-liposome agent was injected into the abdominal lateral cardiac muscle of the heart of myocardiopathy hamster (12 weeks old)

and was subjected to following investigations:

1) Density of blood capillary in the cardiac muscle was measured by ALP (alkaline phosphatase) staining and the result of the HGF gene was compared to that of the control.
2) Bloodstream of the heart to which HVJ-liposome was administered was evaluated by laser Doppler imager (LDI) score and the result of the HGF gene was compared to that of the control.
3) After Masson staining of the cardiac muscle, distribution density of fibrosis was measured by computer analysis. Result of the HGF gene was compared to that of the control.

Reference 1

Preparation of HVJ-liposome agent

[0045] 10 mg Dried lipid (a 1:4.8:2 mixture of phosphatidyl serine, phosphatidyl choline and cholesterol) and 200 μl balanced salt solution (137 μM NaCl, 5.4 μM KCl, 10 μM Tris-HCl; pH7.6) containing HGF gene (100 μg)-HMG1 (high mobility group 1 nuclear protein, 25 μg) was mixed and ,by stirring vigorously with ultrasonication, liposomes were formed. Purified Sendai virus (Z strain) was irradiated with UV (110erg/mm^2/sec) for 3 minutes. Liposome suspension was mixed with Sendai virus (HVJ), heated at 4°C for 10 minutes, and then heated at 37°C for 30 minutes. Free HVJ was discarded and thus obtained HVJ liposome agent.

Reference 2

Measurement on luciferase activity

[0046] Liposome agent with 10 μg of luciferase gene was administered to hamsters (6 animals per group) . A week later, luciferase activity was measured. Results are shown in Figure 1.

[0047] As shown in Figure 1, high levels of luciferase activity were exhibited in the heart. Thus, it was revealed that gene transfer under the usage of echo is possible.

Experiment 1

Treatment of myocardiopathy hamster with HGF gene

[0048] Luciferase agent was injected into the abdominal lateral cardiac muscle of the heart of myocardiopathy hamsters (12 weeks old, 6 animals per group). A group of myocardiopathy hamsters (12 weeks old, 6 animals per group) to which liposome agent containing control vectors was injected in the same manner was used as the control and untreated myocardiopathy hamsters (6 animals per group) were used as the untreated group. Then liposome agents were injected once each week for 8 times. 8 weeks later, density of blood capillary in the cardiac muscle of the heart of the 20 week old myocardiopathy hamsters was measured by ALP staining, and bloodflow was evaluated by the LDI score. After euthanization of the hamsters, the heart was extirpated and after Masson staining, distribution density of fibrosis was measured by computer analysis.

[0049] ALP staining revealed significant rise in blood capillary by angiogenesis in HGF gene treatment group. The results are shown in Figure 2.

[0050] Concerning LDI score, taking the control group as 100%, the HGF gene treatment group was 163±7%, which indicates significant increase in bloodflow. The results are shown in Figure 3.

[0051] According to the analysis of Masson staining, significant decrease in distribution density of fibrosis was observed in HGF gene treatment group. The results are shown in Figure 4.

Industrial Applicability

[0052] Therapeutic agents for myocardiopathy comprising an HGF gene of this invention induce angiogenesis of the affected part of cardiac muscle, increase bloodflow of the affected part while repressing and reducing fibrosis of the cardiac muscle it can repair the cardiac function. Moreover, therapeutic agents of this invention can be injected noninvasively and accurately to the affected cardiac muscle layer visually under the usage of echo. Therefore, therapeutic agents of the invention enable more effective treatment of myocardiopathy.

Claims

1. A therapeutic agent for myocardiopathy used for noninvasive administration comprising a hepatocyte growth factor (HGF) gene as the effective ingredient.

2. The therapeutic agent of claim 1, which is used for administration of the HGF gene into the cardiac muscle.

3. The therapeutic agent of claim 1 or 2, wherein the HGF gene is in the form of Sendai virus (HVJ)-liposome.

4. The therapeutic agent of claim 2 or 3., which is used for noninvasive administration to the affected part of the cardiac muscle under the usage of echo.

5. The therapeutic agent of any of claims 1 to 4, which is to be administered at least 8 times, once a week.

6. The therapeutic agent of any of claims 1 to 5, wherein at least 10 μg of the HGF gene is used.

7. The therapeutic agent of any of claims 1 to 6, where-

in the myocardiopathy is selected from the group consisting of cardiomyopathy, angina pectoris and heart failure

8. A gene therapy agent used for noninvasive administration of a gene into an affected part of a tissue under the usage of echo, which comprises genes effective for the treatment of a disorder as the effective ingredient.

9. The gene therapy agent of claim 8, wherein the affected part of the tissue is the cardiac muscle.

10. The gene therapy agent of claim 8 or 9, wherein the gene is an HGF gene.

11. A method for gene therapy for myocardiopathy, which comprises the noninvasive administration of an HGF gene into the cardiac muscle of a mammal, including a human.

12. The method for gene therapy of claim 11, wherein the HGF gene is in the form of Sendai virus (HVJ)-liposome.

13. The method for gene therapy of claim 11 or 12, wherein the HGF gene is administered noninvasively to a part of an affected cardiac muscle under the usage of echo.

14. The method for gene therapy of any of claims 11 to 13, wherein the HGF gene is administered at least 8 times, once per week.

15. The method for gene therapy of any of claims 11 to 14, wherein the myocardiopathy is selected from the group consisting of cardiomyopathy, angina pectoris and heart failure.

16. A method for gene therapy, which comprises the noninvasive administration of genes effective for the treatment of a disorder into an affected part of a tissue under the usage of echo.

17. The method for gene therapy of claim 16, wherein the affected tissue is the cardiac muscle.

18. The method for gene therapy of claim 16 or 17, wherein the gene is an HGF gene.

19. Use of an HGF gene for the production of a therapeutic agent for myocardiopathy used for noninvasive administration.

20. The use of claim 19, wherein the HGF gene is in the form of Sendai virus (HVJ)-liposome.

21. The use of claim 19 or 20, wherein the therapeutic agent is a therapeutic agent used for the noninvasive administration of the HGF gene to an affected part of the cardiac muscle under the usage of echo.

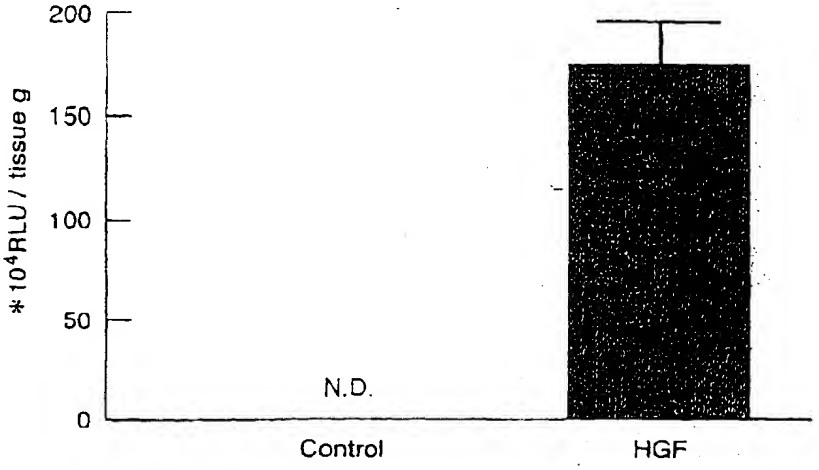
22. The use of any of claims 19 to 21, wherein the myocardiopathy is selected from the group consisting of cardiomyopathy, angina pectoris and heart failure.

23. Use of a gene for the production of a gene therapy agent used for the noninvasive administration of genes effective for the treatment of a disorder into an affected part of a tissue under the usage of echo.

24. The use of claim 23, wherein the affected tissue is cardiac muscle.

25. The use of claim 23 or 24, wherein the gene is an HGF gene.

FIG. 1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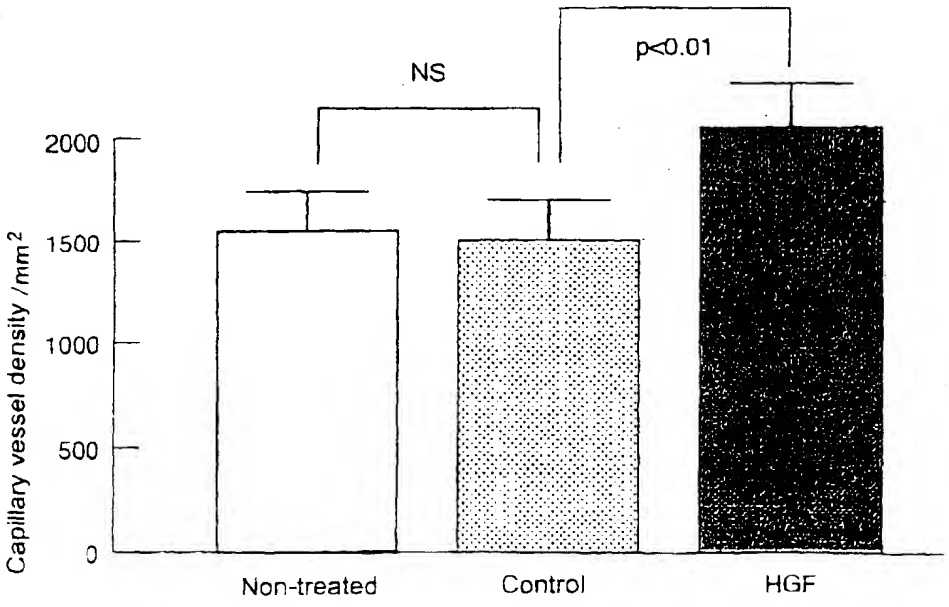
N.D.
Control
HGF

FIG. 2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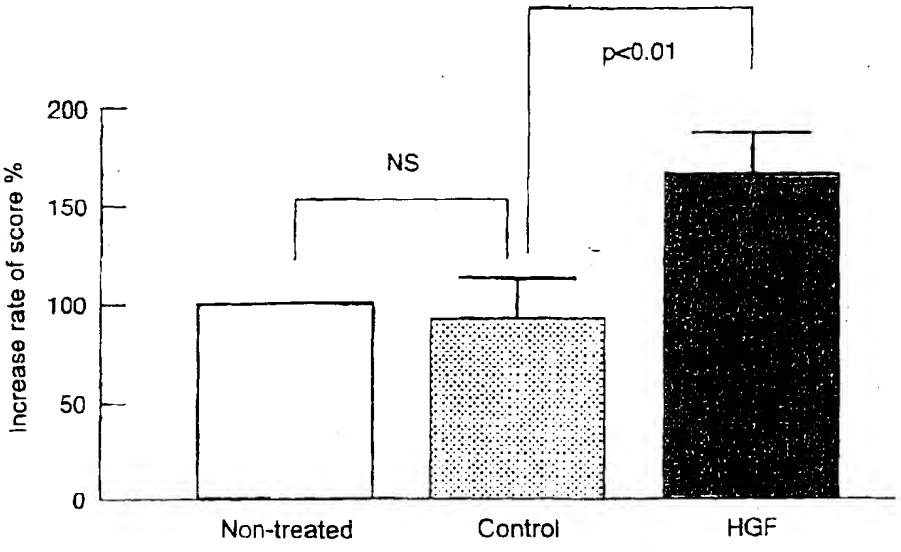
Non-treated
Control
HGF

FIG. 3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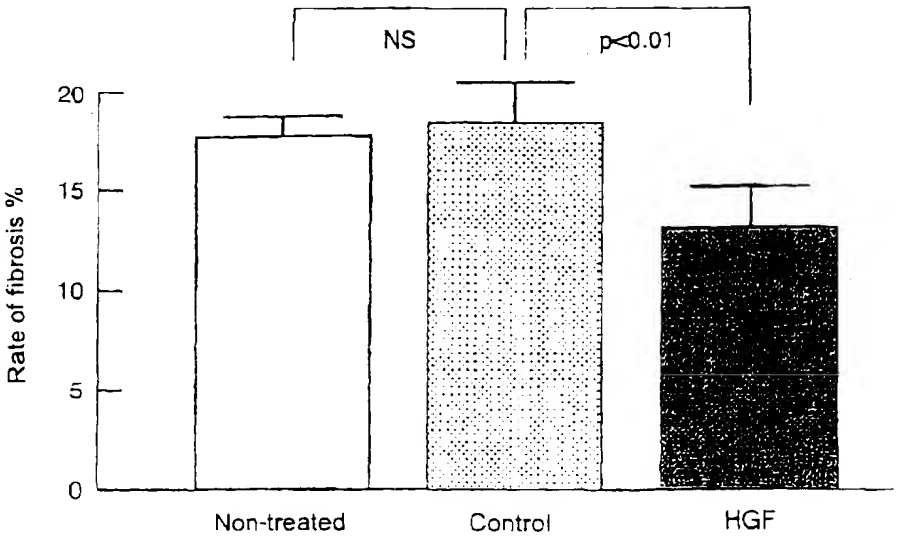
Control
HGF

FIG. 4


NS
p<0.01
20
15
10
5
0
Rate of fibrosis %
Non-treated
Control
HGF

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP00/06947

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] A61K48/00, 38/22, 35/76, 9/127, A61P9/10, 9/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] A61K48/00, 38/22, 35/76, 9/127, A61P9/10, 9/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE(STN), BIOSIS(STN), CA(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	ESAKOF D.D.et al., "Intraoperative multiplane transesophageal echocardiography for guiding direct myocardial gene transfer of vascular endothelial growth factor in patients with refractory angina pectoris", HUMAN GENE THERAPY, (1999) Vol.10, No.14, pp.2307-2314	8,9,23,24 1-7,10, 19-22,25
Y	AOKI, Motokuni et al., "Beneficial angiogenesis induced by over-expression of human hepatocyte growth factor (HGF) in non-infarcted and infarcted myocardium: Potential gene therapy for myocardial infarction", Circulation, (1998) Vol. 98, No. 17, pp.I321	1-7,10, 19-22,25
Y	DERRICK S.G. et al., "Scatter factor induces blood vessel formation in vivo", Proc. Natl. Acad. Sci. USA, (1993) Vol.90, pp.1937-1941	1-7,10, 19-22,25
Y	ERIC Van Belle et al., "Potentiated Angiogenic Effect of Scatter Factor/Hepatocyte Growth Factor via Induction of Vascular Endothelial Growth Factor", Circulation, (1998) Vol.97, pp.381-390	1-7,10, 19-22,25

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 December, 2000 (19.12.00)	26 December, 2000 (26.12.00)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP00/06947

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 11-18
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 11 to 18 pertain to methods for treatment of the human body by surgery or therapy, as well as diagnostic methods, and thus relate to a subject matter which this International Searching Authority is not required to search.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)